desmopressin's safety in this group of healthy Norwegians would translate to the ethically diverse US elderly population, with its multiple medical problems. In addition, acceptable sodium levels after 3 days of treatment does not guarantee that the drug is safe after 1 month or 1 year of administration. Several of my patients have presented to the emergency department with confusion and were subsequently found to have sodium levels less than 125 mmol/L after taking stable does of desmopressin for weeks to months.

Overall, I believe that desmopressin is a safe and effective drug, and I have had success with this treatment in adult neurogenic bladder patients. However, use of desmopressin in the elderly, even if apparently healthy, should be initiated with considerable caution. ■

# Erectile Function

## The Effects of Phosphodiesterase-5 Inhibitors in Men With "Normal" Erectile Function

Reviewed by Jacob Rajfer, MD

*Department of Urology, David Geffen School of Medicine at UCLA; Division of Urology, Harbor-UCLA Medical Center, Los Angeles, CA*

[*Rev Urol.* 2004;6(1):44]



A common belief among the lay population is that oral phosphodiesterase-5 inhibitors will make a "normal" man become "supernormal" regarding his erectile capabilities. Two studies on this topic have been published, both of which have emanated from Italy. In the first study,[1] which was reviewed in a previous issue of *Reviews in Urology*,[2] investigators concluded that sildenafil (50 mg) improved erections in normal men. However, the average age of study subjects was 39 years, an age that impotence experts would most likely not equate with "normal" erectile capabilities.

### Sildenafil Does not Improve Sexual Function in Men Without Erectile Dysfunction but Does Reduce the Postorgasmic Refractory Time

Mondaini N, Ponchietti R, Muir GH, et al.

*Int J Impot Res.* 2003;15:225-228.

In the second study, by Mondaini and colleagues, the investigators lowered the threshold of normal age to a mean age of 33 years in the sildenafil group (n = 30) and 31 years in the placebo group (n = 30). The International Index of Erectile Function (IIEF) score was 26 or greater at baseline for all subjects in both groups. Patients received sildenafil, 25 mg, or placebo and recorded their posttreatment IIEF score and refractory time to obtaining a second erection.

Study results demonstrated no difference in erectile function between the subjects who received sildenafil and those who received placebo. However, the subjects who received sildenafil recorded a lower refractory time than those who received placebo ($P < .04$). A criticism of this study is that the subjects received only 1 pill, so that outcome measures were made after only 1 trial. Nevertheless, this study suggests that sildenafil will not make a normal man supernormal in terms of his erection but may reduce the refractory time in young men. Whether it is appropriate for a physician to treat a patient with the goal of improving latency time is debatable. ■

**References**

1. Rochira V, Granata AR, Balestrieri A, et al. Effects of sildenafil on nocturnal penile tumescence and rigidity in normal men: randomized, placebo-controlled, crossover study. *J Androl.* 2002;23:566-571.
2. Rajfer J. Prescribing PDE5 inhibitors: who is the "normal" man? *Rev Urol.* 2002;4:197-198.

# Prostate Cancer

## Tissue Microarrays in Prostate Cancer Research

Reviewed by Masood A. Khan, MD, Alan W. Partin, MD, PhD

*The Johns Hopkins Hospital, Baltimore, MD*

[*Rev Urol.* 2004;6(1):44-46]



The high-density tissue microarray (TMA) was developed by Kononen and colleagues[1] for the purpose of rapidly analyzing a large number of samples with either in situ or immunohistochemical methods on a single slide. The technique involves taking core tissue biopsies (diameter, 0.6 mm; height, 3-4 mm) from individual "donor" paraffin-embedded tumor blocks and arraying them in a new "recipient" paraffin block (45 × 20 mm) using a custom-built instrument. Using 0.6-mm cores while preserving the histologic information allows as many as 1000 specimens to be arrayed in a single recipient

block (single slide) with minimal damage to the original tissue blocks. Up to 200 consecutive tissue sections (4-8 μm) can be cut onto individual slides from each block array. Serial sectioning of the blocks allows rapid, parallel analyses of the arrayed tumor punches by several methods, including immunohistochemistry, fluorescence in situ hybridization, and RNA/RNA in situ hybridization.

The major advantage of TMA is that analysis of tumors from many different patients with different stages of disease can be performed simultaneously. This not only saves considerable time but also dramatically reduces expenditure and improves intra-sample testing reliability. However, the major drawback of this technique is that, because of the small punch size, TMAs may not demonstrate tumor heterogeneity, which can commonly be estimated in whole section mounts. Therefore, the choice of the tumor area is pivotal and, in the case of widely heterogeneous tumor, numerous punches may be necessary. The following studies report on the use and limitations of TMAs in prostate cancer research.

*The major advantage of TMA is that analysis of tumors from many different patients with different stages of disease can be performed simultaneously.*

## Tissue Microarray Sampling Strategy for Prostate Cancer Biomarker Analysis

**Rubin MA, Dunn R, Strawderman M, et al.**

*Am J Surg Pathol.* 2002;26:312-319.

Because prostate cancer is a heterogeneous tumor, the use of TMAs for clinical biomarker studies may be of limited value. However, in an attempt to overcome this disadvantage, the authors investigated the optimal TMA sampling size needed to accurately identify prostate cancer biomarkers. To this end, prostate cancer proliferation as determined by Ki-67 immunohistochemistry was studied. (Ki-67 is a nuclear protein that is expressed in G1, S, G2, and M phases of the cell cycle but not in the G0 phase [at rest].[2]) Ten replicate measurements of proliferation using digital image analysis were made on 10 regions of prostate cancer from a standard glass slide. Five matching TMA sample cores were tested from each of the 10 regions in the parallel study. A bootstrap resampling analysis was used to statistically simulate all possible permutations of TMA sample number per region or sample. Statistical analysis compared TMA samples with Ki-67 expression in standard pathology immunohistochemistry slides.

The optimal sampling for TMA cores was reached at 3, as fewer samples significantly increased Ki-67 variability and a larger number did not significantly improve accuracy. To validate these results, a prostate cancer outcomes TMA containing 10 replicate tumor samples from 88 cases was constructed. Similar to the initial study, 1 to 10 randomly selected cores were used to evaluate the Ki-67 expression for each case, computing the ninetieth percentile of the expression from all samples used in each model. Using this value, a Cox proportional hazards analysis was performed to determine predictors of time until prostate-specific antigen (PSA) recurrence after radical prostatectomy for clinically localized prostate cancer. Examination of multiple models demonstrated the optimal number of cores to be 4. Using a model with 4 cores, a Cox regression model demonstrated that Ki-67 expression, preoperative PSA, and surgical margin status predicted time to PSA recurrence with hazard ratios of 1.49 (95% confidence interval [CI], 1.01-2.20; $P < .05$), 2.36 (95% CI, 1.15-4.85; $P < .05$), and 9.04 (95% CI, 2.42-33.81; $P < .01$), respectively. Models with 3 cores to determine Ki-67 expression were also found to predict outcome. The authors, therefore, concluded that 3 cores are required to optimally represent Ki-67 expression with respect to the standard tumor slide. Furthermore, 3 to 4 cores provided the optimal predictive value in a prostate cancer outcomes array and should be useful in evaluating other putative prostate cancer biomarkers.

## Inadequate Formalin Fixation Decreases Reliability of p27 Immunohistochemical Staining: Probing Optimal Fixation Time Using High-Density Tissue Microarrays

**De Marzo AM, Fedor HH, Gage WR, et al.**

*Hum Pathol.* 2002;33:756–760.

$p27^{Kip1}$ is a cyclin-dependent kinase inhibitor that is expressed nearly uniformly in the prostate luminal secretory cells.[3] It is downregulated in carcinoma and has been proposed as a potential biomarker of which immunohistochemical detection may be useful in predicting prognosis.[3-5] The authors had previously noted that, with standard formalin fixation in rapidly processed (same-day) radical prostatectomy specimens, there was often a gradient of $p27^{Kip1}$ staining in normal prostate epithelium, with more staining near the periphery and less toward the center (unpublished observations). This observation raised the hypothesis that the reliability of staining for $p27^{Kip1}$ might be reduced in inadequately fixed tissues. This hypothesis

was tested using 2 TMAs containing 564 tissue samples. Results demonstrated that there was a significant increase in the percent of cores that stained strongly for $p27^{Kip1}$ as fixation time increased from 0 (same-day processing) to 1 or more days ($P < .001$). The authors, therefore, concluded

*Brief tissue fixation to decrease diagnostic turnaround time might limit the reliability of interpretation of some forms of immunohistochemical staining.*

that brief tissue fixation to decrease diagnostic turnaround time might limit the reliability of interpretation of some forms of immunohistochemical staining. In addition, and more importantly, TMAs, which assure identical test conditions, provide an excellent platform for the evaluation of the effects of tissue fixation on immunohistochemical staining.

### Limitations of Tissue Microarrays in the Evaluation of Focal Alterations of bcl-2 and p53 in Whole Mount Derived Prostate Tissues

**Merseburger AS, Kuczyk MA, Serth J, et al.**

*Oncol Rep.* 2003;10:223–228.

Several investigators have reported the correlation of p53 and bcl-2 immunoreactivity with postoperative PSA recurrence.[6-8] Focal and/or clustered expression is typical for these biomarkers. The purpose of this study was to compare the effectiveness of TMAs to detect p53 and bcl-2 overexpression and their prognostic significance. TMAs of 99 patients, with a mean follow-up of 61 months, contained 760 samples from 241 carcinomas, 431 benign glands, and 88 foci of prostatic intraepithelial neoplasia (PIN). Through the use of TMA technology, overexpression of p53 and bcl-2 was detected in 43.3% and 23.7% of the patients, respectively, compared with 66.0% and 26.9% in the corresponding radical prostatectomy samples. Therefore, although TMA is regarded as a powerful tool to study the multifocal and heterogeneous nature of prostate cancer, the prognostic value of p53 and bcl-2 could not be confirmed using this technology in contrast to radical prostatectomy sections. To this end, TMA is probably more informative and reliable in evaluating the prognostic value of homogeneously expressed biomarkers.

In conclusion, TMAs have a great advantage in that numerous tissues can be investigated at the same time, which not only reduces time and cost but also assures identical test conditions for all the samples. However, the limitation of TMAs appears to be their relative inability to demonstrate heterogeneity of the tumor because of the small sample size used. ■

**References**


1. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med.* 1998;4:844-847.
2. Gerdes J, Lemke H, Baisch H, et al. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol.* 1984;133:1710-1715.
3. Guo Y, Sklar GN, Borkowski A, Kyprianou N. Loss of the cyclin-dependent kinase inhibitor p27(Kip1) protein in human prostate cancer correlates with tumor grade. *Clin Cancer Res.* 1997;3(12 pt 1):2269-2274.
4. Yang RM, Naitoh J, Murphy M, et al. Low p27 expression predicts poor disease-free survival in patients with prostate cancer. *J Urol.* 1998;159:941-945.
5. Cote RJ, Shi Y, Groshen S, et al. Association of p27Kip1 levels with recurrence and survival in patients with stage C prostate carcinoma. *J Natl Cancer Inst.* 1998;90:916-920.
6. Oxley JD, Winkler MH, Parry K, et al. p53 and bcl-2 immunohistochemistry in preoperative biopsies as predictors of biochemical recurrence after radical prostatectomy. *BJU Int.* 2002;89:27-32.
7. Cesinaro AM, Migaldi M, Ferrari G, et al. Expression of p53 and bcl-2 in clinically localized prostate cancer before and after neo-adjuvant hormonal therapy. *Oncol Res.* 2000;12:43-49.
8. Stackhouse GB, Sesterhenn IA, Bauer JJ, et al. p53 and bcl-2 immunohistochemistry in pretreatment prostate needle biopsies to predict recurrence of prostate cancer after radical prostatectomy. *J Urol.* 1999;162:2040-2045.


## Vasectomy and Prostate Cancer


**Reviewed by Masood A. Khan, MD, Alan W. Partin, MD, PhD**

*The Johns Hopkins Hospital, Baltimore, MD*


[*Rev Urol.* 2004;6(1):46-47]



Vasectomy is the most frequently used form of male contraception in the United States, with approximately 500,000 procedures performed annually.[1] However, several case-control and cohort studies conducted over the past decade have demonstrated conflicting results regarding the possible association between vasectomy and prostate cancer risk.[2-5] This has raised considerable concern, not only among men undergoing vasectomy but also among urologists performing the procedure. Many urologists now screen for prostate cancer early in men who have had a vasectomy and even discourage vasectomy in men with a strong family history of prostate cancer.[6]

The following study further investigated the possible association between vasectomy and prostate cancer in New Zealand, which has the highest prevalence of vasectomy in the world.[7]

### Vasectomy and Risk of Prostate Cancer

**Cox B, Sneyd MJ, Paul C, et al.**

*JAMA.* 2002;287:3110-3115.

The authors conducted a national population-based case-control study of 923 new cases of prostate cancer among